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Polyacetylenes in Related Thistles of the Subtribes Centaureinae and Carduinae

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Key Word Index—*Centaurea*; *Cirsium*; *Cynara*; *Carthamus*; *Carduus*; *Acroptilon*; Centaureinae; Carduinae; Asteraceae; thistles; polyacetylenes; systematics.

Abstract—The distribution of polyacetylenes in eight closely related thistles belonging to the subtribes Centaureinae and Carduinae (Asteraceae) was examined and showed similar patterns among the annuals of each subtribe. The perennial in each subtribe, i.e. Russian knapweed (*Acroptilon repens*) and globe artichoke (*Cynara scolymus*) both contained more polyacetylenes than the annuals, and the subtribe Centaureinae had the greatest number of, and the more complex, polyacetylenes.

Introduction

Polyacetylenes and their thiophene addition products are a large group of compounds found predominantly in the family Asteraceae [1] and often show a wide variety of biological activities including phytotoxicity [2], larvicidal [3–5], insecticidal, nemoticidal and fungicidal [3, 6–8] properties. In many instances these and other properties are enhanced by exposing the test compounds to sunlight or UV radiation. Their phototoxic activity against a number of plant pests such as phytopathogenic fungi [9, 10], nematodes [8] and herbivorous insects [6, 11] strongly suggests that these compounds play a key role as natural protective agents in the thistle family.

Weeds introduced into the United States, such as yellow starthistle (*Centaurea solstitialis* L.) and Russian knapweed (*Acroptilon repens* L.), often invade crop- and rangeland unchecked because, when introduced to a new continent, they usually leave their adapted enemies/herbivores behind, and herbivores, e.g. phytophagous insects, native to their new area of naturalization have not adapted to the chemical constituents and other factors found in the non-native plant species. Thus, in order to control these noxious weeds, host specific insects from points of origin are imported and screened for non-adaptability on native or commercially valuable closely related plant species. Since polyacetylenes have been implicated as protective agents in the thistle tribe (Asteraceae, Cardueae), it may be important to determine the distribution of these compounds in closely related species, thus allowing the correlation with insect susceptibility and/or resistance.

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Results

The distribution of polyacetylenes in the roots for each of the species investigated is shown in Table 1. The most notable aspect is the large number of thiophene analogs (**4–16**) found in the roots of Russian knapweed (*Acroptilon repens* L.) which are not found in the other species. However, roots of all the species contained compounds **1** and **2**, with five and four conjugated acetylene units, respectively. With the exception of these two compounds, douglas thistle (*Cirsium douglassi* DC.) and musk thistle (*Carduus nutans* L.) have no detectable polyacetylenes in their roots. Occidental thistle (*Cirsium occidentale* Nutt) is very similar, with the exception of compound **19**. Even though Russian knapweed contains a large number of thiophene derivatives of polyacetylenes it does not contain any of the compounds **17–26**. This is in stark contrast with the composition of the roots for safflower (*Carthamus tinctorius* L.), purple starthistle (*Centaurea calcitrapa* L.), globe artichoke (*Cynara scolymus* L.) and yellow starthistle (*Centaurea solstitialis* L.).

In general, fewer polyacetylenes were found in the leaves than were found in the roots (Table

TABLE 1. DISTRIBUTION OF POLYACETYLENES IN ROOTS OF RELATED THISTLES*

	Centaureinae				Carduinae			
Compound	A†	B	C	D	E	F	G	H
1	x	x	x	x	x	xx	xxx	xx
2	x	xxx	xxx	xxx	xxx	x	xx	x
3	x	x						
4	x							
5	x							
6	x							
7	xxx							
8	xxx							
9	xx							
10	x							
11	x							
12	x							
13	x							
14	x							
15	x							
16	x							
17			x‖					
18		x‡	xxx‖	x§	x			
19							x§	
20		x	x					
21		x§	xxx§	xxx	xxx§			
22		x	x	x‖				
23		x§	x					
24								
25								
26		x			x			

*xxx Relatively large amount of polyacetylene; x relatively small amount of polyacetylene.

†A *A. repens*, B *C. solstitialis*, C *C. calcitrapa*, D *C. tinctorius*, E *C. scolymus*, F *C. douglassi*, G *C. occidentale*, H *C. nutans*.

‡Four isomers were detected.

§Two isomers were detected.

‖Three isomers were detected.

TABLE 2. DISTRIBUTION OF POLYACETYLENES IN LEAVES AND FLOWERS OF RELATED THISTLES*

	Leaves					Flowers			
Compound	A†	B	C	D	E	A	B	C	D
1	xxx		x	x	x	x	x	x	x
2	xxx	x	x	x		xxx	xx	x	
3						x			
4									
5			x					x	
6			x			x		x	
17	xx		x						
18	x	x	x‡					x	
19			x	x‡				x‡	
20							xx	x	
21	x‡	xx‡				xxx‡	xx	x	
22						x‡	xxx‡	x	
23		xxx§					xxx§	x	

*xxx Relatively large amount of polyacetylene detected; x relatively small amount of polyacetylene.

†A *C. scolymus*; B *C. tinctorius*; C *C. calcitrapa*, D *C. occidentale*; E *C. douglassi*.

‡Two isomers detected.

§Three isomers detected.

2). Artichoke leaves were very similar to its roots with respect to the composition; however, the quantity of material is substantially less. Quite surprisingly, the leaves and stems of safflower do not contain detectable quantities of compound **1** even though it is found in the roots. This substance is found in almost all members of the family Asteraceae [1]. Safflower also contained fewer straight chain polyacetylenes in the aerial parts than in the roots.

Of the flowers investigated (Table 2) for polyacetylenes, artichoke showed the largest number of compounds, even exceeding the number found in the roots. Both safflower and purple starthistle flowers showed substantial numbers of polyacetylenes. However, their distribution was somewhat different, i.e. safflower contained compounds **1, 2, 20–23** while purple starthistle contained compounds **1, 2, 5, 6, 18–23**. Occidental thistle flowers are practically devoid of polyacetylenes, containing only the pentayne compound **1**.

Discussion

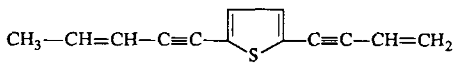
Matricaria ester (**27**), phenyl heptatriyne (**28**) and α-terthienyl (**29**) have all been shown to possess biological activity and in particular insecticidal activity. However, these compounds were not found in the present investigation. McLachlan *et al.* [12] studied the structure–activity relationship among several polyacetylenes and found that, with an increasing number of thiophene groups, the activity increased. Extending this correlation to the present study, Russian knapweed should be the most unacceptable to all types of predators, a phenomenon substantiated by field observations, i.e. Russian knapweed appears to be highly resistant to all types of native pathogens.

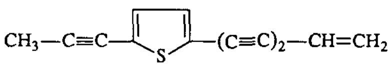
In attempts to control yellow starthistle via the importation of predators from European sources, the most likely candidate for crossover of the insect to non-target plant species would be safflower and purple starthistle. This conclusion is based on the observation that the distribution and number of polyacetylenes in yellow starthistle, safflower and purple starthistle are very similar.

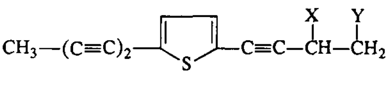
1 $CH_3-(C\equiv C)_5-CH=CH_2$

2 $CH_3-CH=CH-(C\equiv C)_4-CH=CH_2$

3 $CH_3-(C\equiv C)_3-(CH=CH)_3-H$

4 

5 



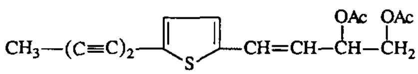
6 X=Y=O (epoxide)

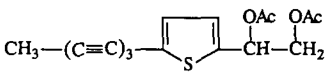
7 X=Cl; Y=OAc

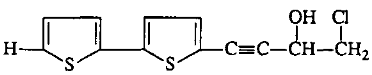
8 X=OAc; Y=Cl

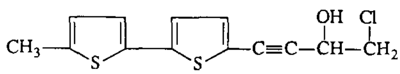
9 X=OH; Y=Cl

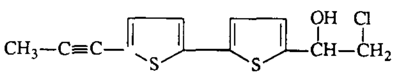
10 X=OAc; Y=OAc

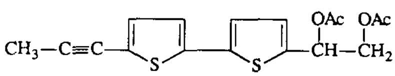
11 

12 

13 

14 

15 

16 

17 $CH_3-CH=CH-(C\equiv C)_2-(CH=CH)_2-CH_2-CH_3$

18 $CH_3-CH=CH-(C\equiv C)_2-(CH=CH)_2-\overset{X}{C}H-\overset{Y}{C}H_2$

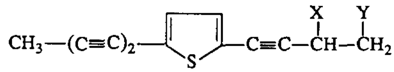
19 $CH_3-(C\equiv C)_3-(CH=CH)_2-\overset{X}{C}H-\overset{Y}{C}H_2$

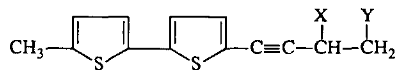
20 $CH_3-(CH=CH)_2-(C\equiv C)_4-H$

21 $CH_3-CH=CH-(C\equiv C)_3-(CH=CH)_2-H$

22 $CH_2=CH-(CH=CH)_2-(C\equiv C)_2-CH=CH-CH_2X$

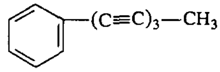
23 $CH_3-CH=CH-(C\equiv C)_3-CH=CH-\overset{X}{C}H-\overset{Y}{C}H_2$

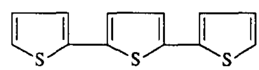
24 

25 

26 $CH_3-(C\equiv C)_4-CH=CH-\overset{X}{C}H-\overset{Y}{C}H_2$

27 $CH_3-CH=CH-(C\equiv C)_2-CH=CH-COOCH_3$

28 

29 

The eight thistles investigated belong to the tribe Cardueae but are from two subtribes. Russian knapweed, yellow starthistle, safflower and purple starthistle belong to the subtribe Centaureinae. Globe artichoke, douglas thistle, musk thistle and occidental thistle all belong to the subtribe Carduinae. Examination of the roots (Table 1) reveals that the largest number of, as well as the more complex, polyacetylenes in the subtribe Centaureinae are found in the perennial, *viz.* Russian knapweed. Likewise, the greater number of polyacetylenes in the subtribe Carduinae are found in the only perennial, *viz.* globe artichoke. The annuals and biennials in each group appear to be very similar. For instance, yellow starthistle (annual), safflower (annual) and purple starthistle (annual/biennial) from the subtribe Centaureinae are very similar in polyacetylene content. These data are consistent with their placement in the same subtribe. Also, douglas thistle (biennial), musk thistle (biennial) and occidental thistle (biennial) have very similar polyacetylene patterns, again confirming their placement in the same subtribe. It thus becomes apparent that two factors correlate with the content and distribution of polyacetylenes in the thistles investigated, namely their taxonomy and their life cycle.

Experimental

Plant material. Whole plants of yellow starthistle (*C. solstitialis* L.) and purple starthistle (*C. calcitrapa* L.) were collected on 20 July 1987 from wild weedy populations at Wild Horse Canyon Ranch near Napa, Napa county, California. Douglas thistle (*Cirsium douglassi* DC.) was collected on 23 July 1987 at Stewart Springs near Edgwood, Siskiyou county, California, among a natural population in moist soil. Whole plants of globe artichoke (*Cynara scolymus* L.) were collected on 15 May 1987 at the University of California Gill tract, Albany, Alameda county, California. The leaves and roots of musk thistle (*Carduus nutans* L.) were collected on 21 May 1987 near Mt Shasta, Siskiyou county, California. Whole plants of safflower var. 555 (*Carthamus tinctorius* L.), were collected on 3 June 1987 near Woodland, Yolo county, California. Whole plants of occidental thistle (*Cirsium occidentale* Nutt) were collected on 16 June 1987 near Abbotts lagoon, Point Reyes peninsula, Marin county. Whole plants of Russian knapweed (*Acroptilon repens* L.) were collected on 24 March 1987 near Discovery Bay, Contra Costa county, California.

Extraction and analysis. Freshly collected plants were divided into roots, leaves and stems and each of the parts ground in a commercial Waring blender with dichloromethane. The slurry was allowed to stand at room temperature (24 h) in the dark, then filtered. Concentration of the filtrates in a rotary evaporator at $< 30^\circ C$ in subdued light gave concentrates which were then filtered through a reverse phase Sep–Pac (Waters Associates) column and analysed by high pressure liquid chromatography (HPLC) using a diode array detector. A reverse phase column (C-18, 25 cm $\times$ 4.1 mm) was used to separate the components with water (22.5%)/methanol (77.5%) as the eluting solvent. Elution of the components ranged from 10 to 55 min and were identified by comparison of their retention times with authentic samples [14] and analysis of their UV spectra [1].

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